Minimal Residual Disease (MRD) as a Surrogate Endpoint in ALL
FDA Workshop, 18 April 2012, Silver Spring, MD

EuroFlow Consortium and MRD strategies

Novel concept in diagnostic flow cytometry

Jacques J.M. van Dongen
on behalf of

EuroFlow

Achievements of the EuroFlow Consortium

EuroFlow

Immunobeads
- special immunobead assay for detection of fusion … in leukemias
- multiplex approach for fusion protein detectio… category

Multicolor flow cytometry (≥8 colors) wit… …ndardization
- inclusion of violet laser and sele… …te fluorochromes
- standardization of instrume… …boratory protocols
- detailed testing and co… …ody clones and conjugated antibodies (multiple companie…

Implementati… …ent of novel software
- fast a… …g of large data files (including automated pattern recognition)
- c… …tubes: calculation and APS view
- ma… …agnosis and follow-up leukemia samples against templates of "norm… …ontrol" samples

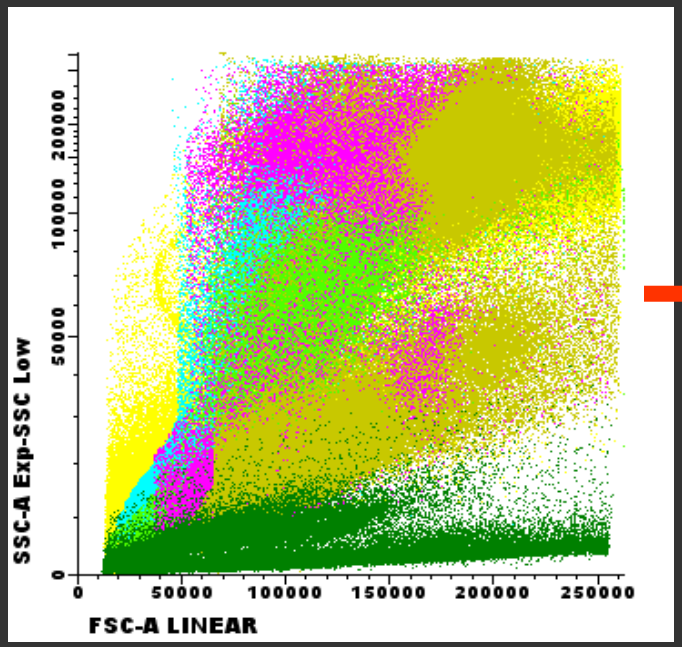
Full standardization

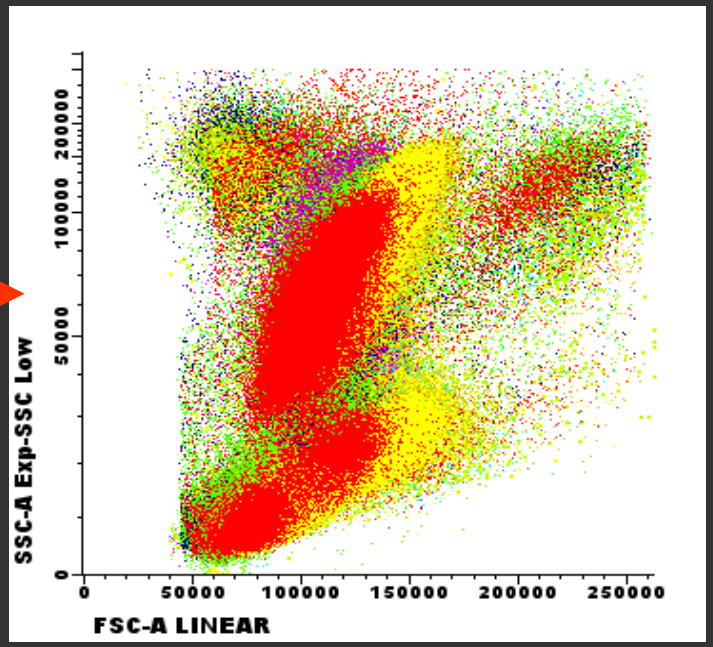
Development of 8-color antibody protocols for diagnosis, classification and monitoring of hematological malignancies
- screening tubes (include recognition of normal leukocyte subsets)
- multi-tube panels for diagnosis and classification per disease category
- special tubes for MRD monitoring per disease category

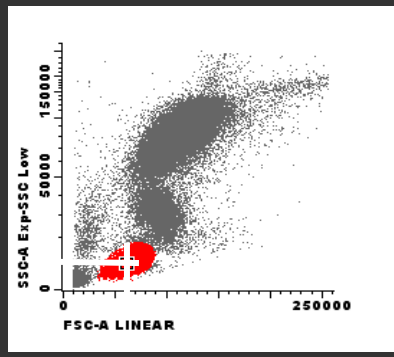
Synchronized light scatter experiments

"Local" settings

EuroFlow settings







7 different normal PB samples acquired in 7 different centers

Normal PB samples processed according to
the standardized EuroFlow sample preparation protocol

EuroFlow

Immunophenotypic classification & identification of LAIP

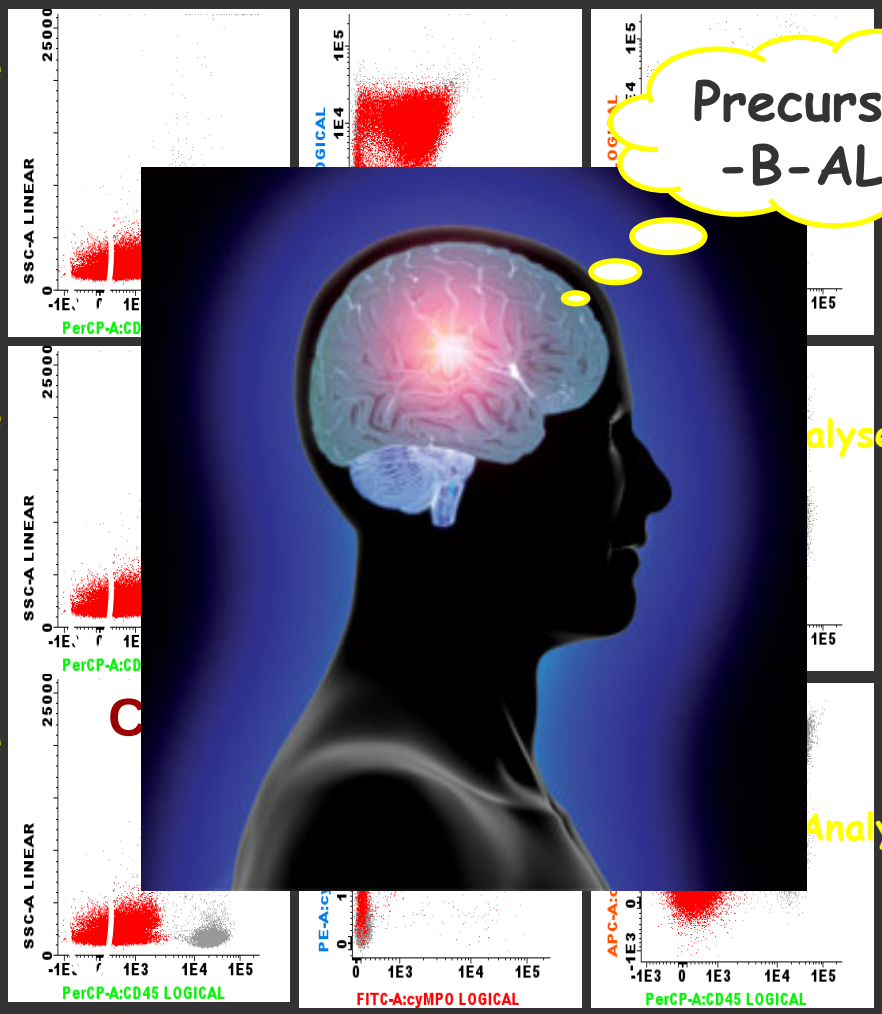
EuroFlow

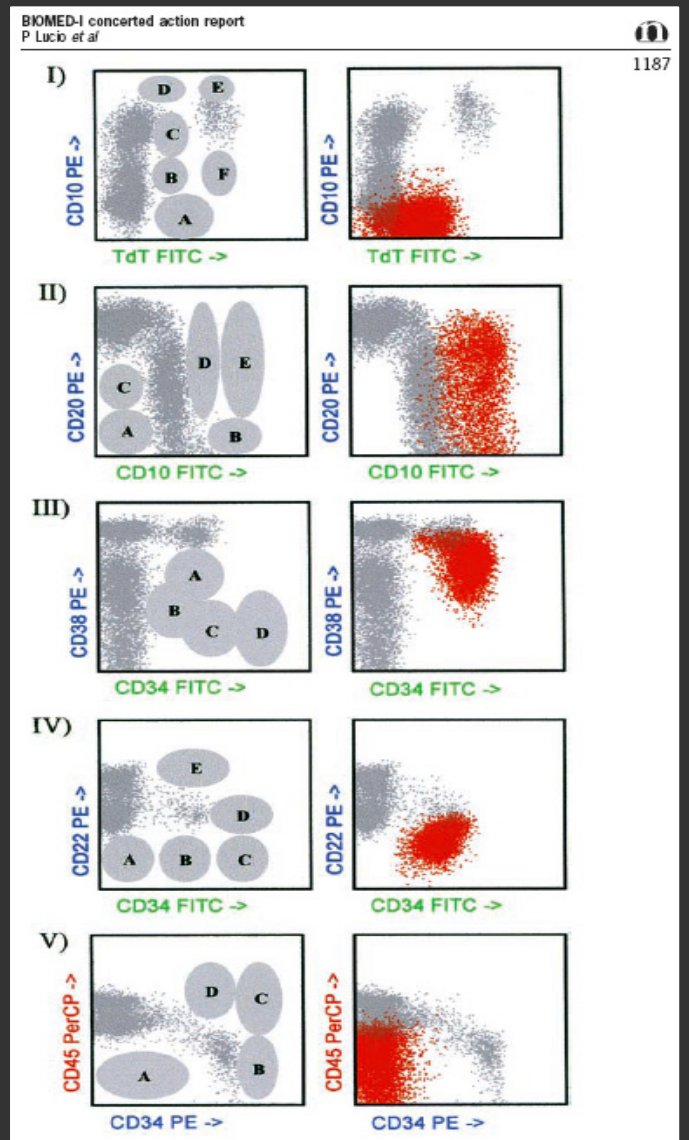
TdT+ / CD19+ / CD38+

Tube

Tube

Tube





JJM van Dongen Department of Immunology, Erasmus MC

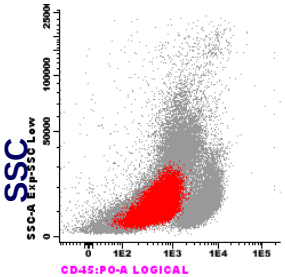
IMMUNOPHENOTYPIC CHARACTERISTICS OF NORMAL vs LEUKEMIC B-CELLS

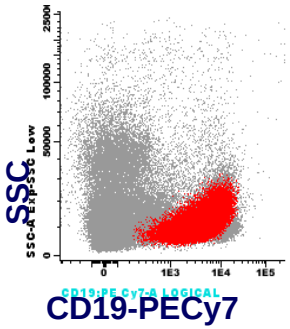
EuroFlow

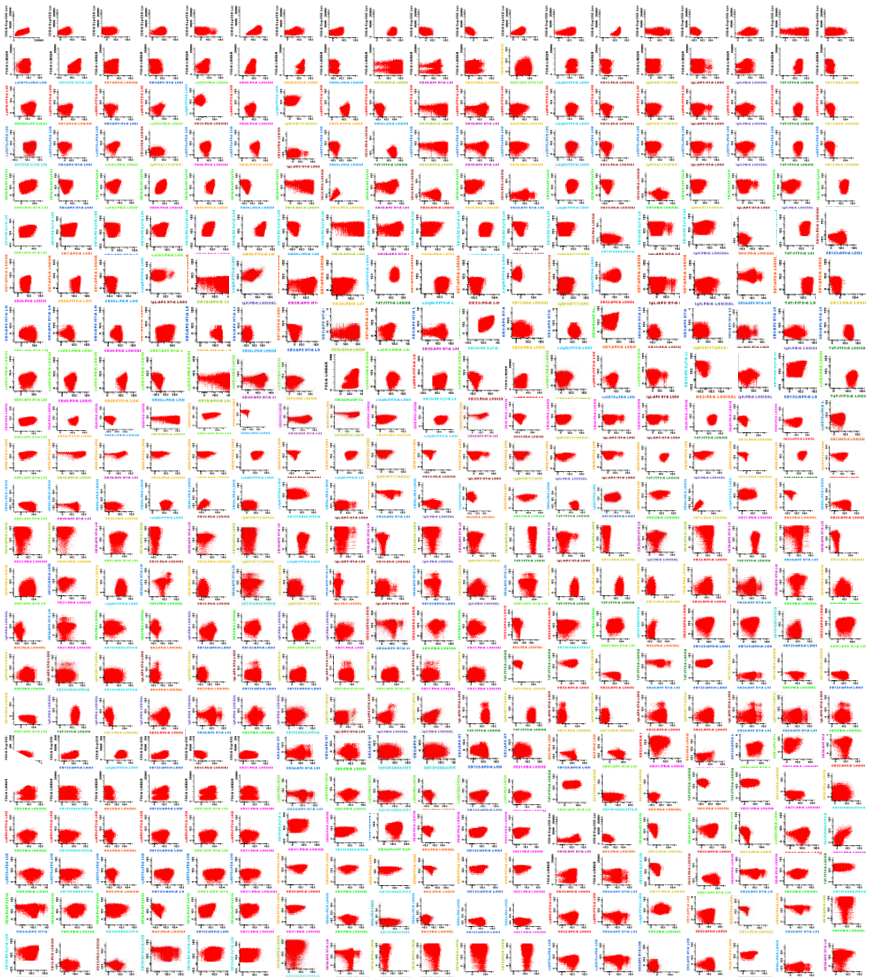
CD19+
B-CELLS

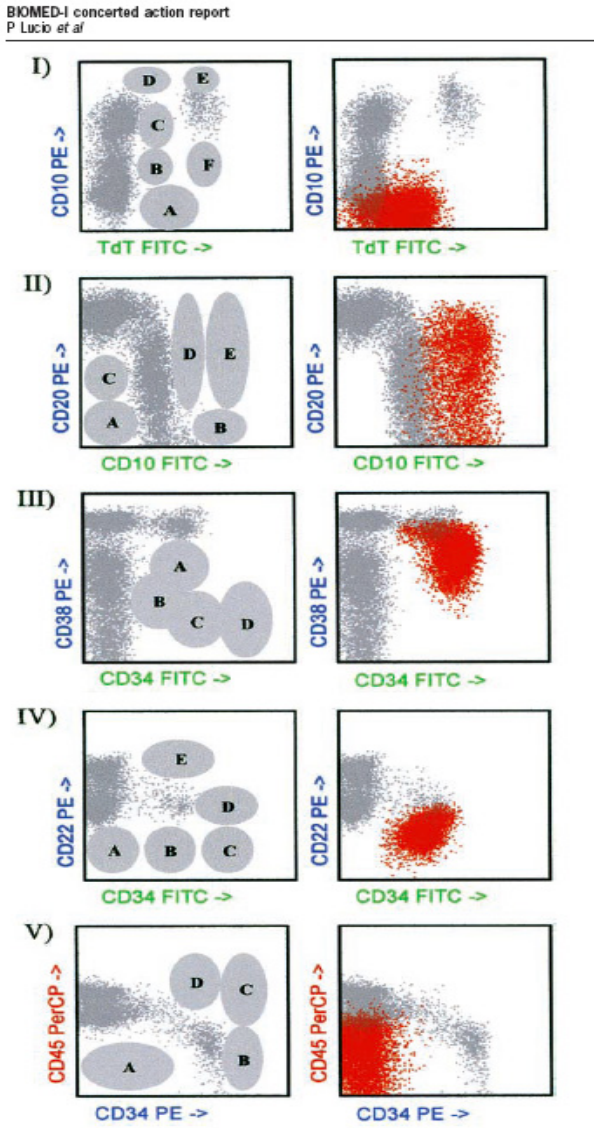
8- COLOR flow cytometry: BCP-ALL

EuroFlow panel (450 bivariate plots)







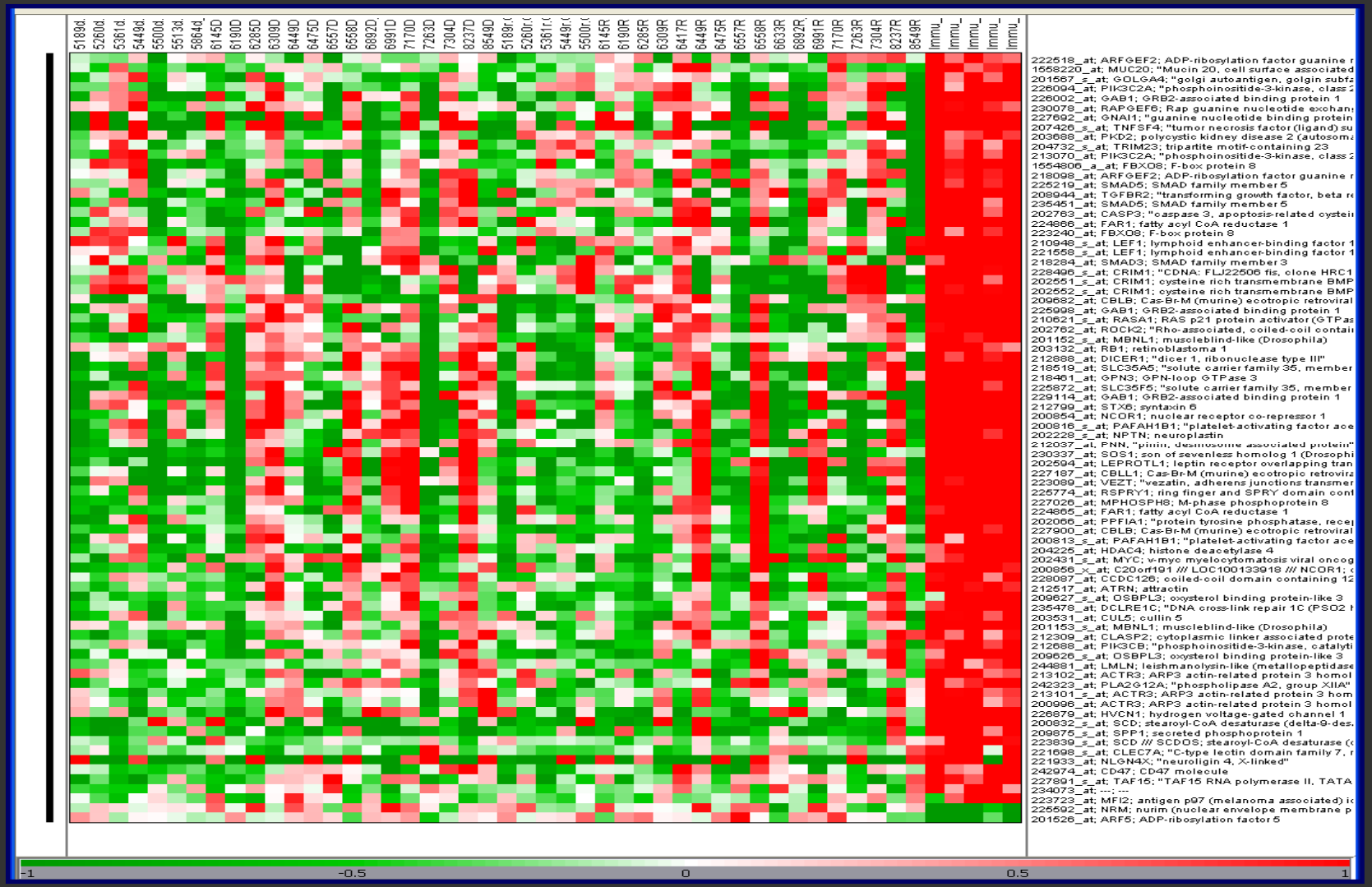


Lucio et al, Leukemia, 1999

Gene expression profiling of ALL cells in BM at diagnosis and purified ALL cells at extramedullary sites

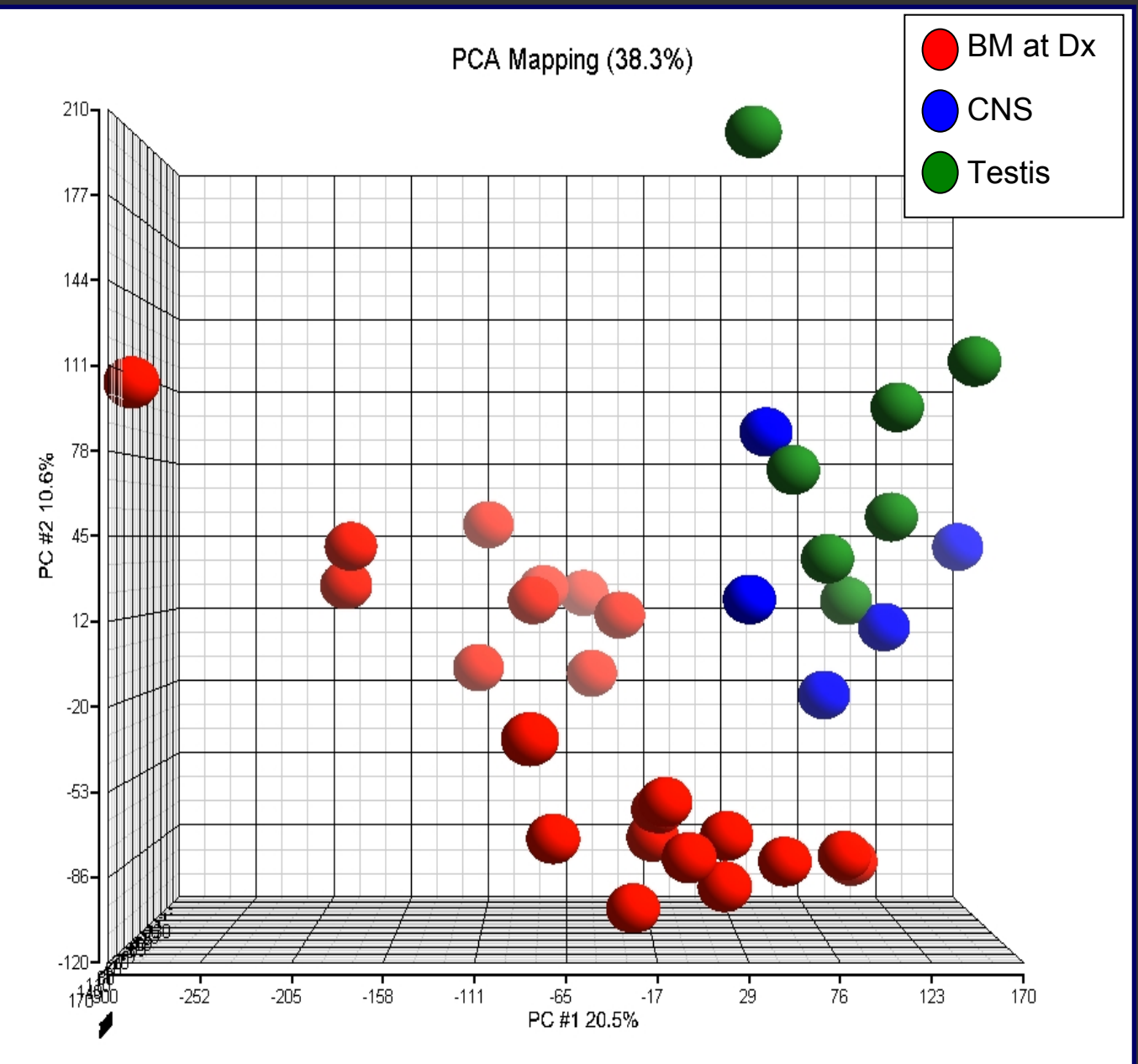
Diagnostic BM samples of relapsed patients (n=22)

CNS samples (n=5)



Heat map of gene expression

Gene expression profiling of ALL cells in BM at diagnosis and purified ALL cells at extramedullary sites



PCA study
(Principal Component Analysis)

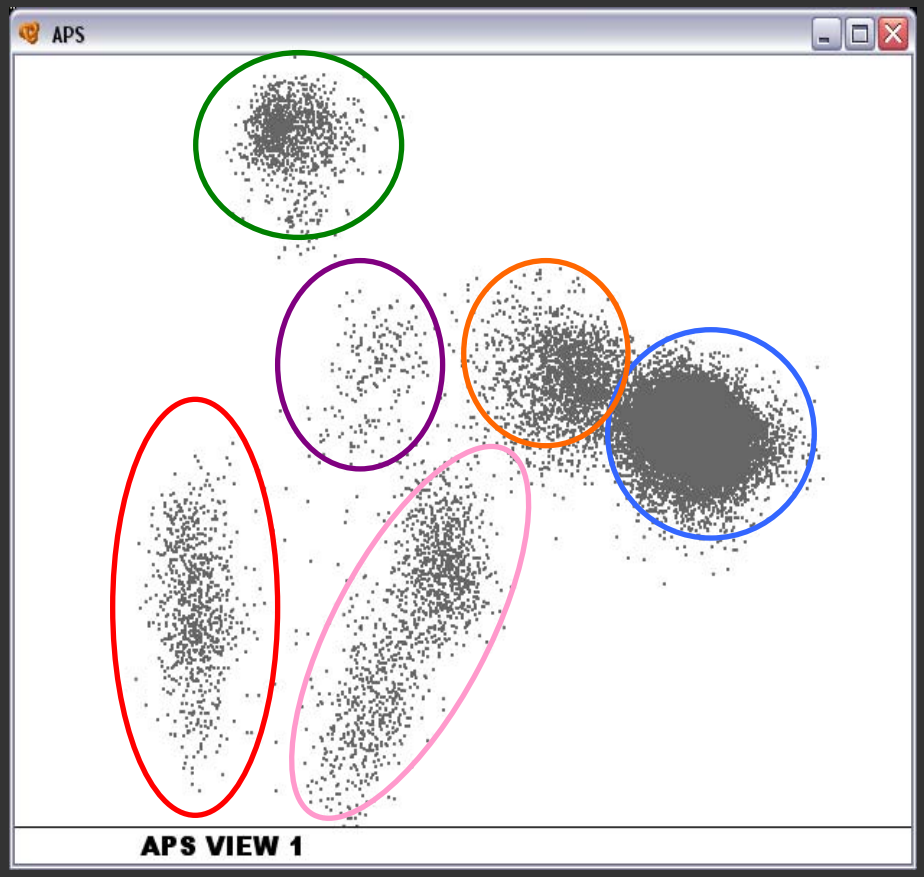
V.H.J.van der Velden
& J de Vries,
unpublished results

Automatic identification of populations

EuroFlow

Multidimensional analysis:

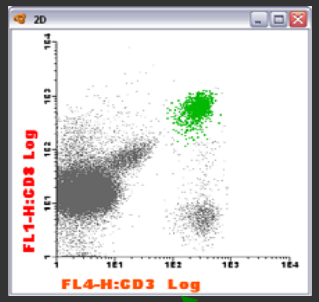
Automated Separation among different cell Populations (APS view)

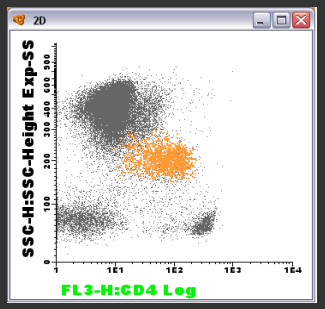


Automatic identification of populations

EuroFlow

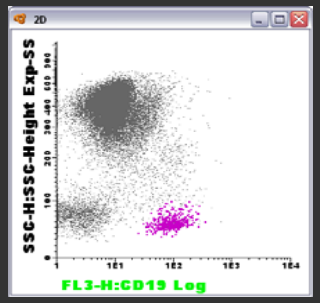
CD8+ T-lymfocytes

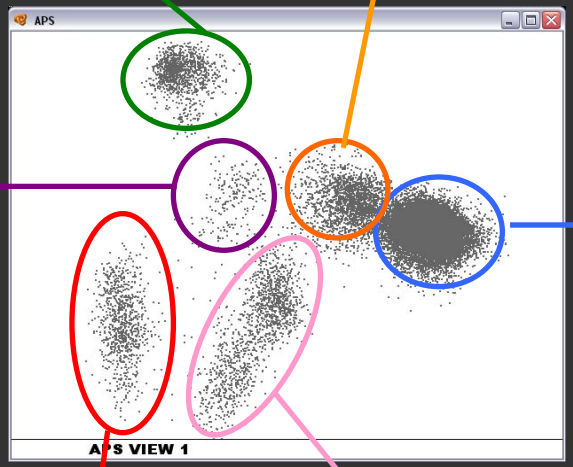


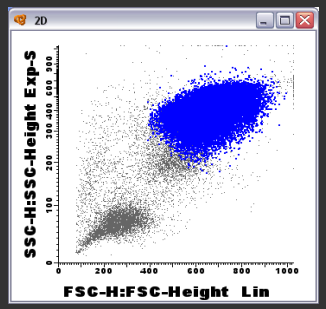


Monocytes

B-cells

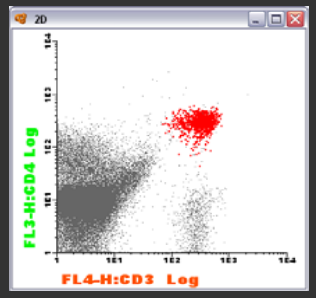


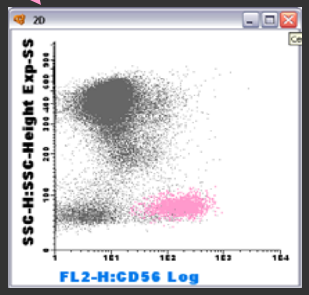




Neutrophils

CD4+ T-lymfocytes



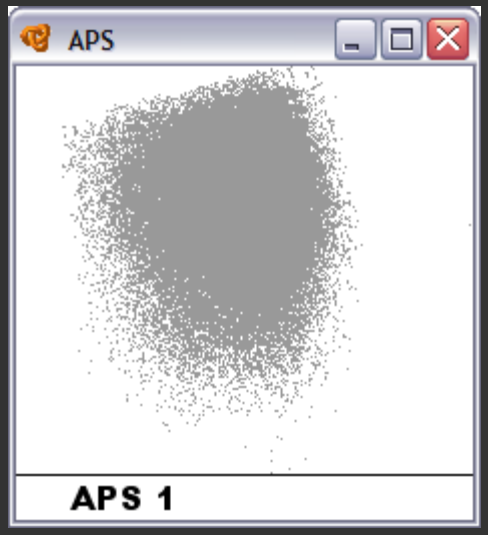


NK-cells

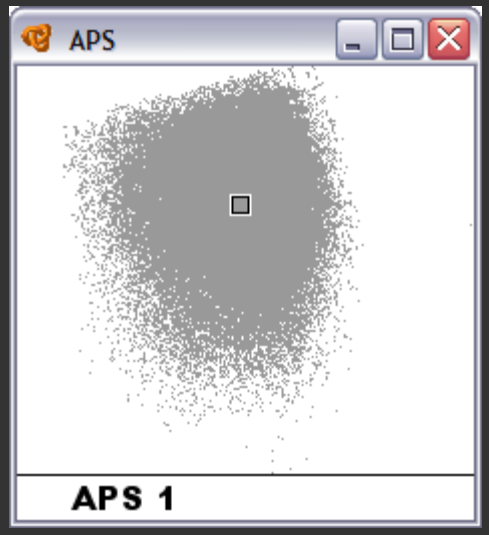
www.infinicyt.com

APS Procedure for AUTOMATIC ANALYSIS

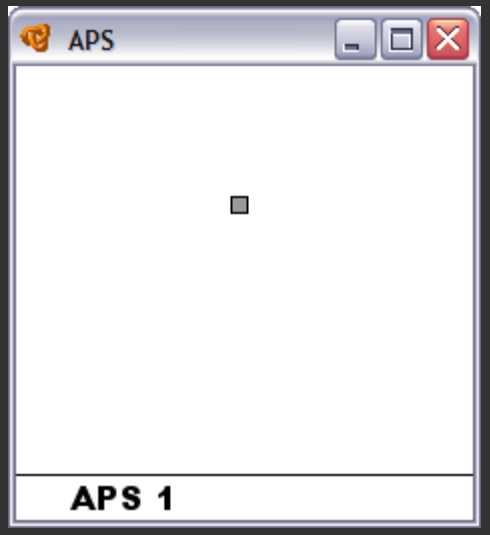
VISUALIZATION OPTIONS



Dots

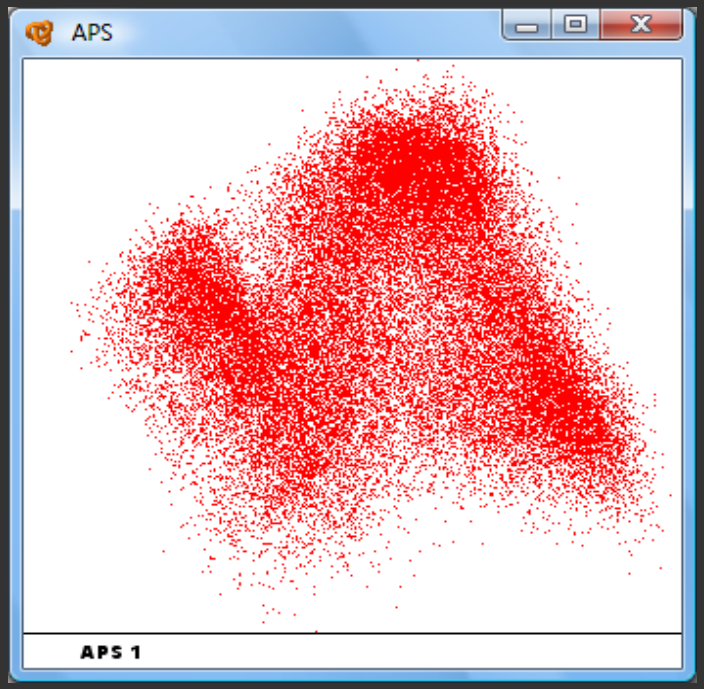


Dots / Mean

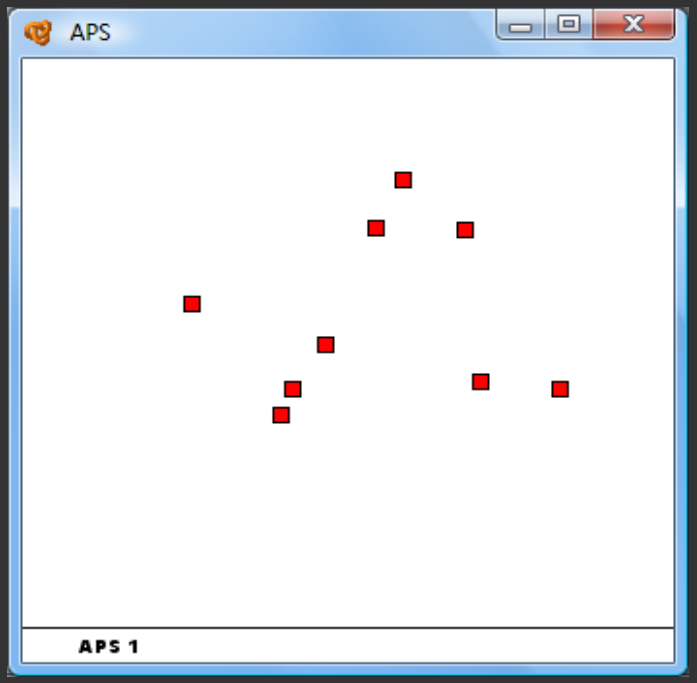


Mean

APS Procedure for groups of patients



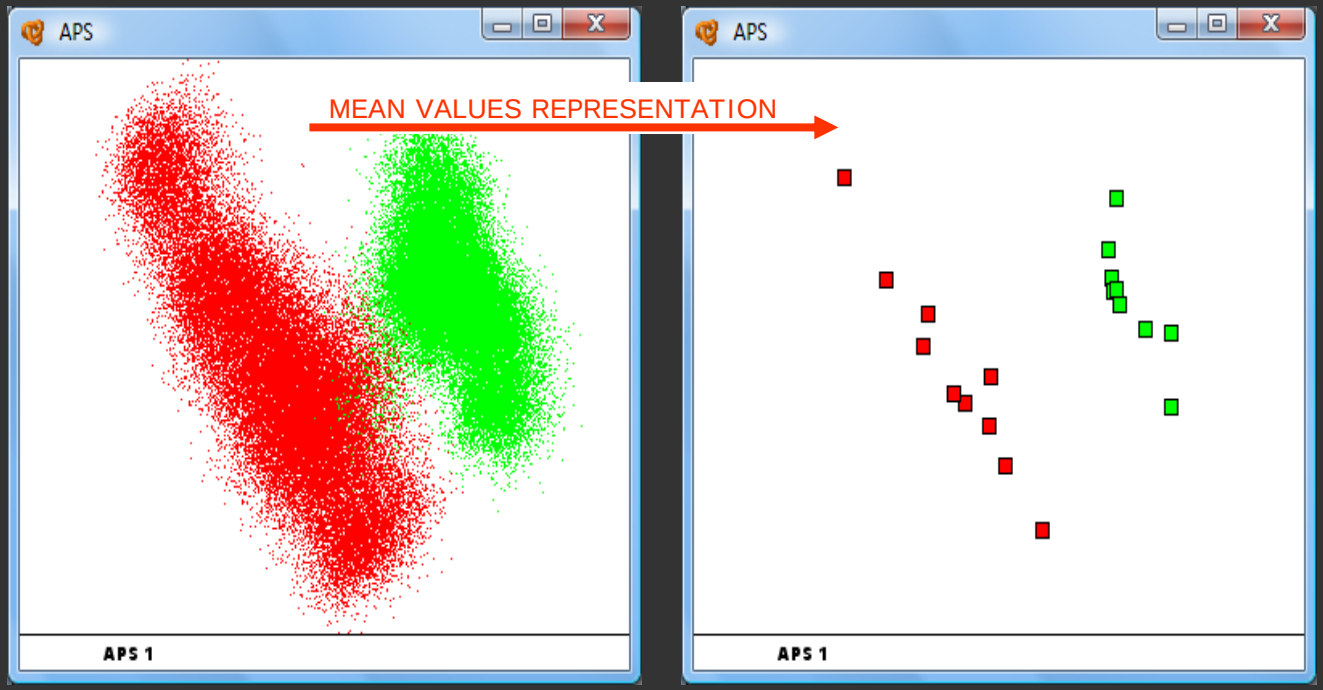
APS Dots View



APS Means View

Group of patients with the same panel/protocol applied and same disease category

APS Procedure for groups of patients



Each dot

=

patient/sample

Group of patients with same panel/protocol applied and 2 different disease categories

Results of synchronized experiments

APS view of 30 merged data files from different centers

CD14+ Monocytes

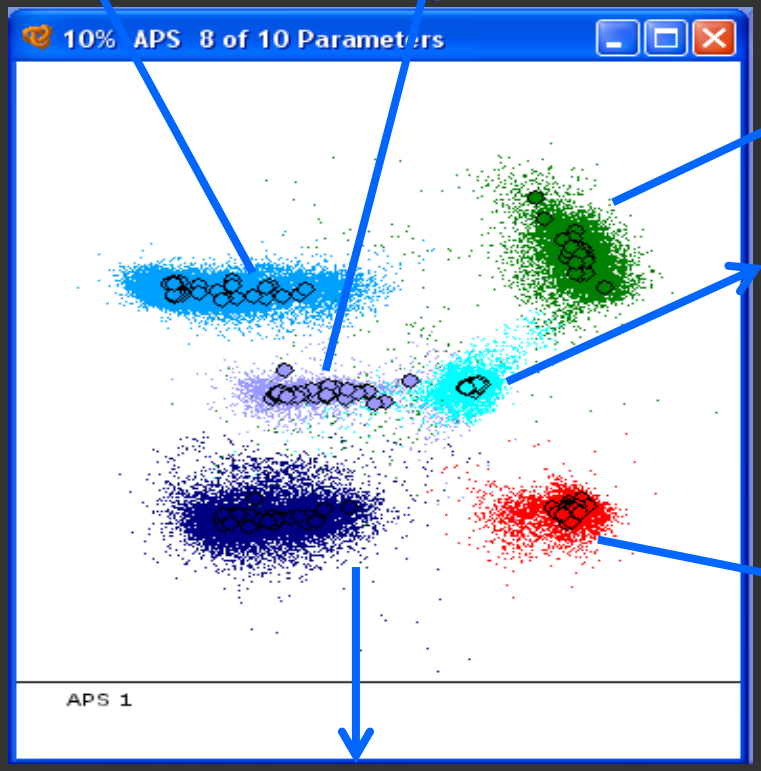
CD8+ CD3- T cells

CD3+ CD4+ T-cells

CD3+ CD8- CD4- T cells

CD3+ CD8+ T cells

CD19+ CD20+ B-cells

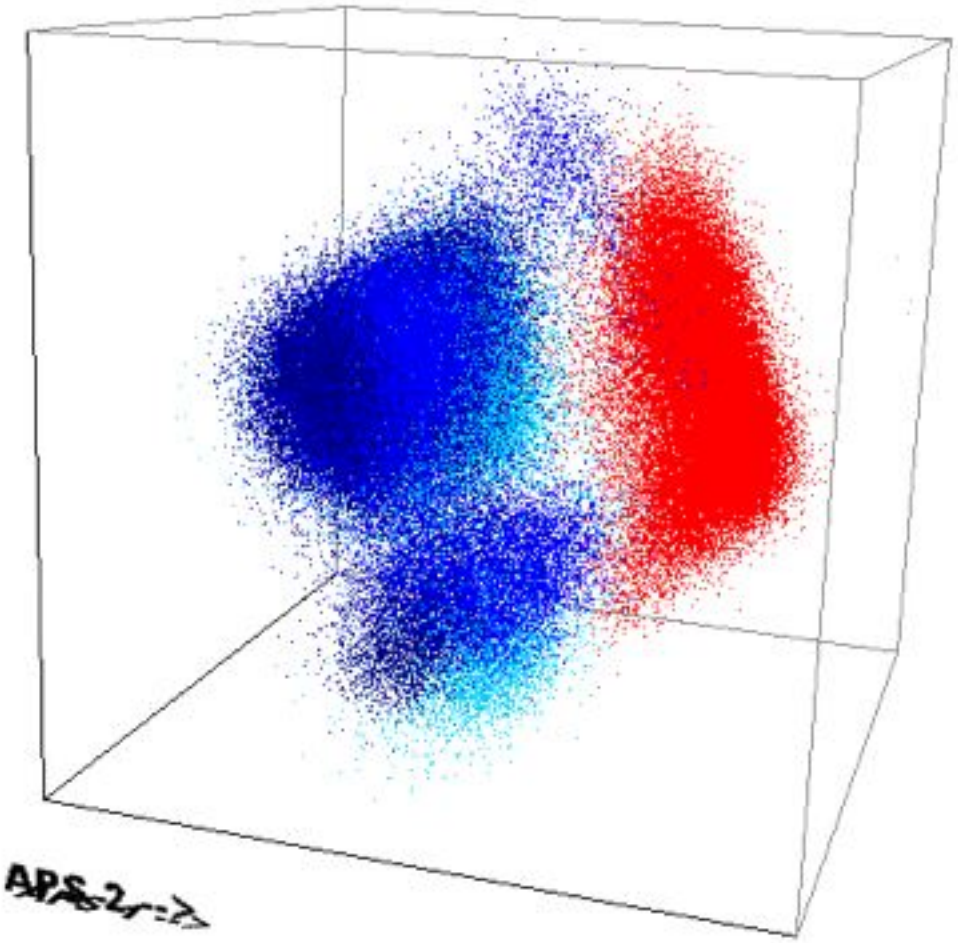


Local normal donors

CV MFI subset

PacBlue channel	Lym - CD20pos	16,90%
PacOr channel	Lym - CD45 pos	15,52%
FITC channel	Lym - CD3+ CD8pos	16,93%
PE channel	Lym - CD3+ CD27pos	27,95%
PerCPCy55 chann	Lym - CD3+ CD4pos	28,39%
PECy7 channel	Lym - CD19 pos	15,40%
APC channel	CD45pos - CD14 pos	22,68%
APC-H7 channel	Lym - CD3 pos	**48,35%**

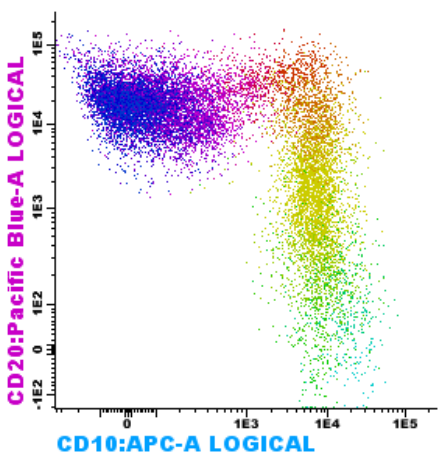
EuroFlow

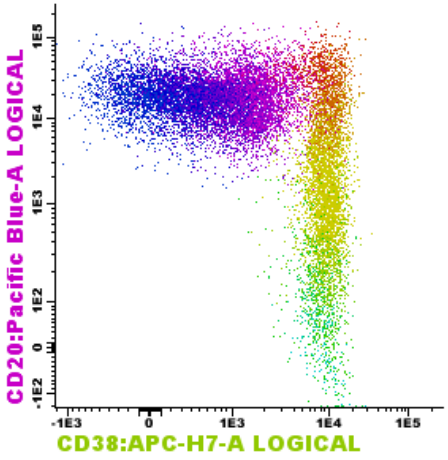


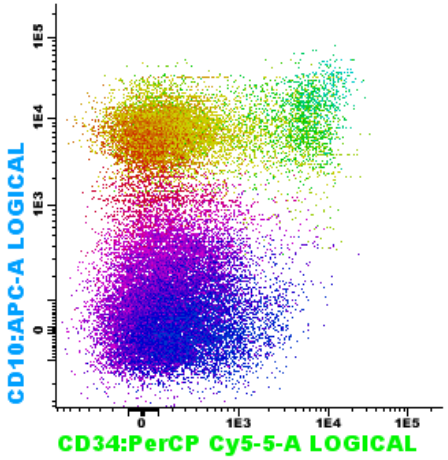
EuroFlow

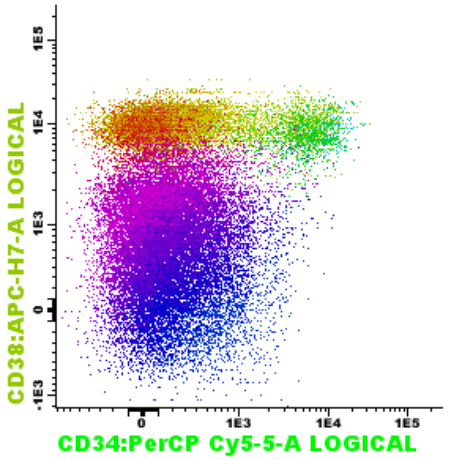
Responsible scientist: L Lhermitte

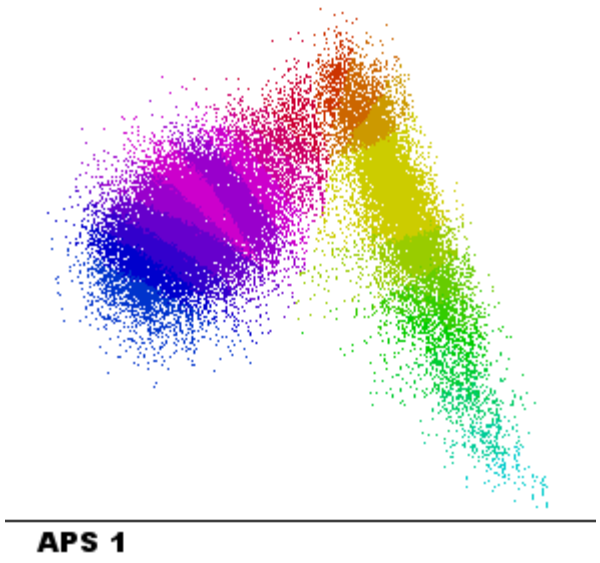
Dissection of normal precursor-B-cell differentiation

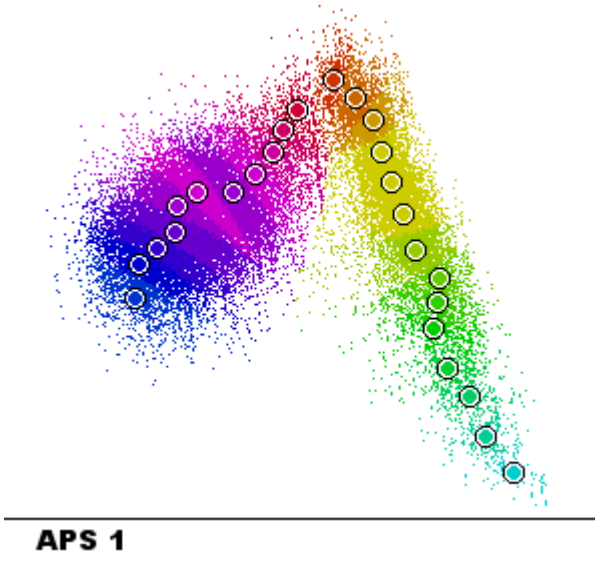


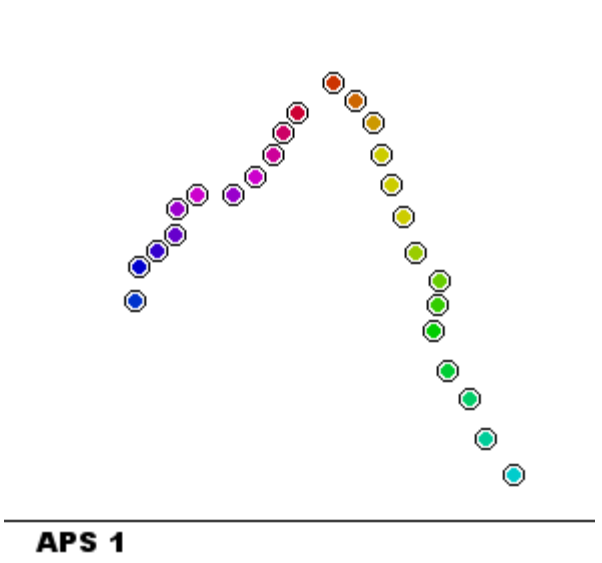






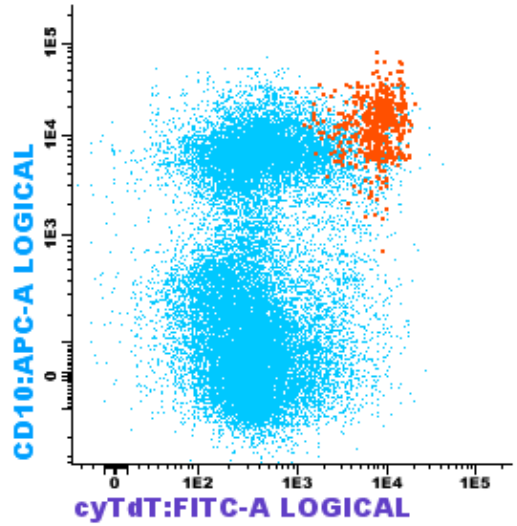


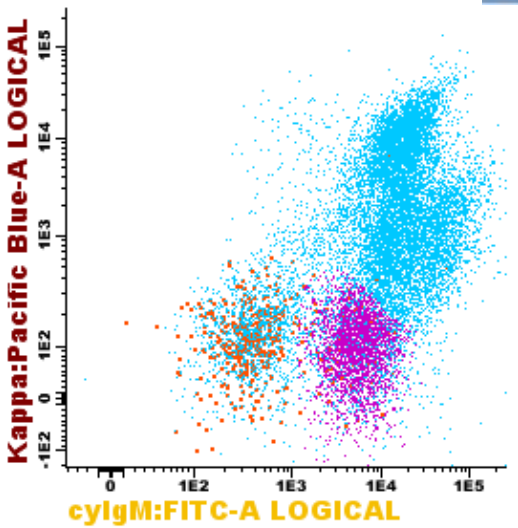


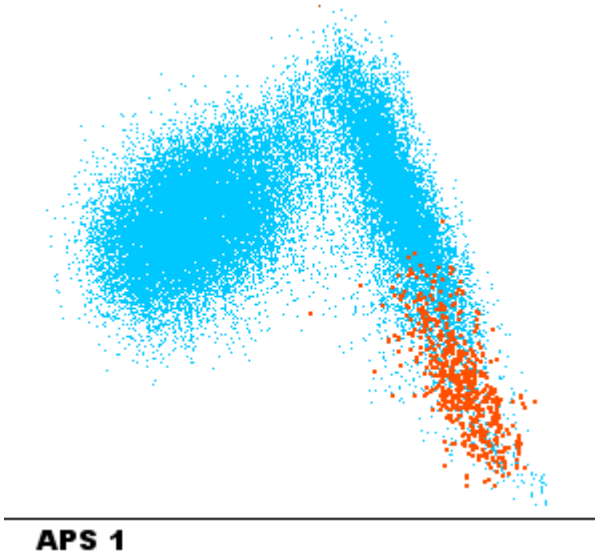


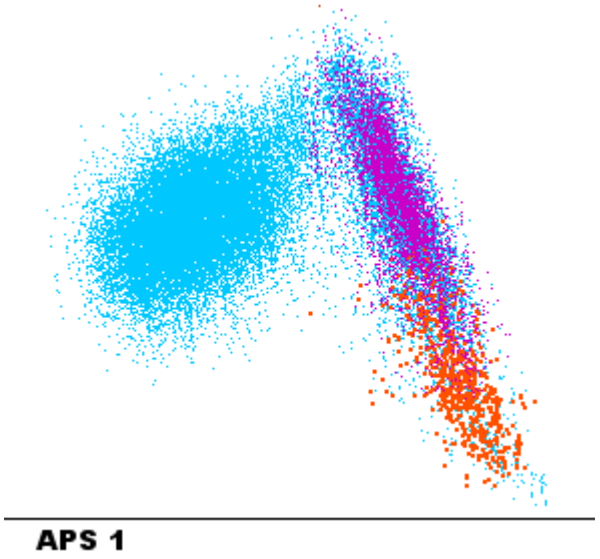
Responsible scientists: V.H.J. van der Velden and E. Mejstrikova

Dissection of normal precursor-B-cell differentiation





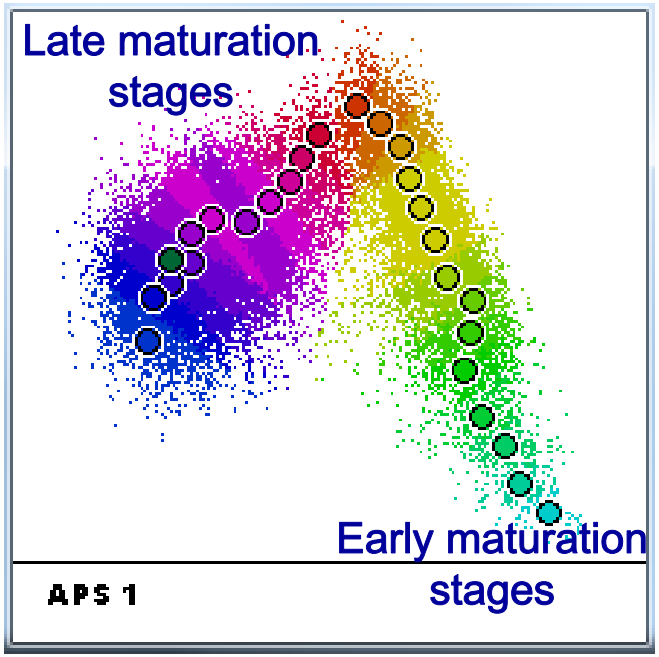




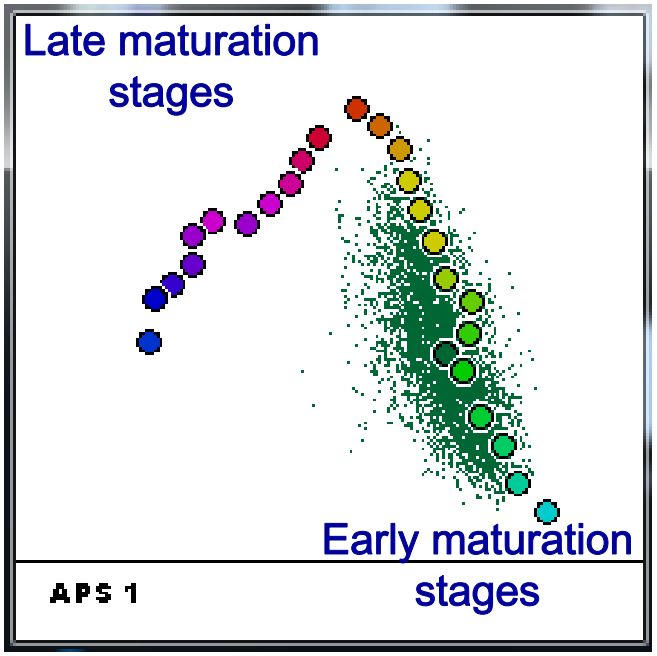
Responsible scientists: V.H.J. van der Velden and E. Mejstrikova

Precursor B-cell differentiation in normal vs regenerating bone marrow

Normal BM



Normal vs Regenerating BM



CD19 Gated B-cells (excluding PC)

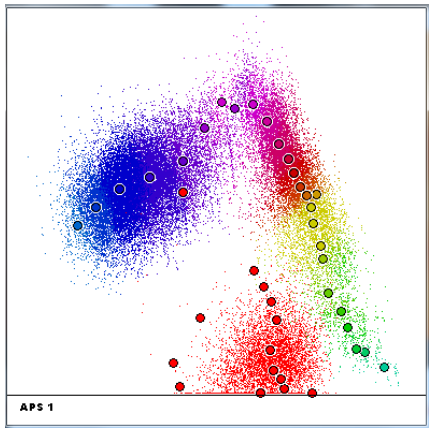
Responsible scientists: V.H.J. van der Velden and E. Mejstrikova

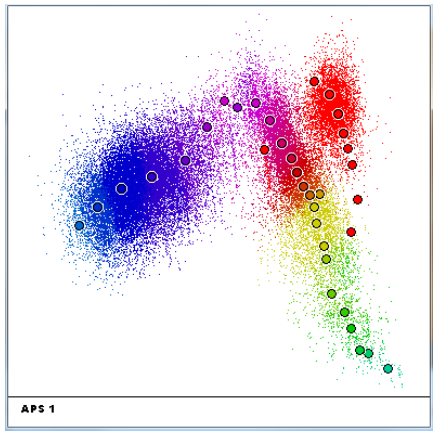
EuroFlow

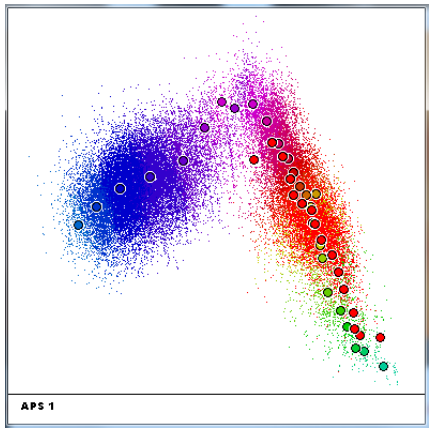
Four BCP-ALL cases vs normal precursor B-cells

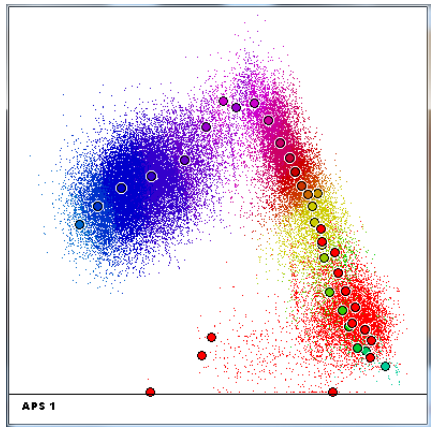
EuroFlow

APS view 1

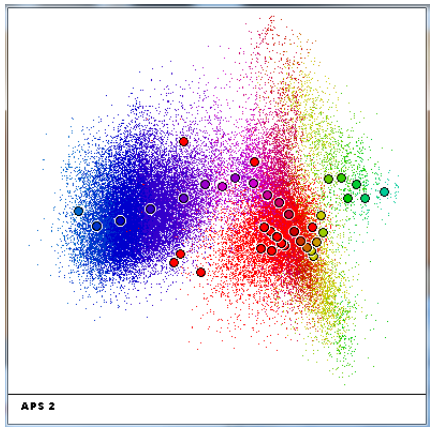


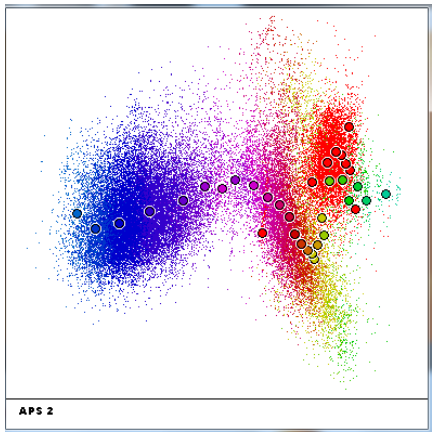


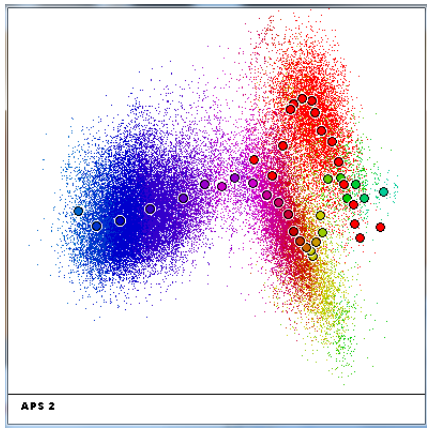


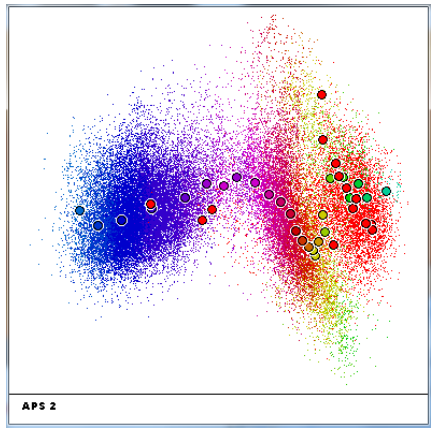


APS view 2









Case 1 Case 2 Case 3 Case 4

Responsible scientists: V.H.J. van der Velden and E. Mejstrikova

Designed by: A. Orfao & Q Lecrevisse

Development of 8-color MRD panels

EuroFlow

Single-tube antibody EuroFlow MRD protocols under evaluation

1. Acute leukemias (includes recognition of normal precursors)
   - Acute myeloid leukemia panel (AML-MRD): 1 tube per pathway (A. Orfao)
   - B-cell precursor (BCP-ALL-MRD): 1 tube (V. van der Velden, E. Mejstrikova)
   - T-cell ALL (T-ALL-MRD): 1 tube (V.Asnafi)

2. Chronic lymphoproliferative disorders (includes recognition of normal cells)
   - Chronic lymphocytic leukemia (CLL-MRD): 1 tube (A. Langerak)
   - Hairy cell leukemia (HCL-MRD): 1 tube (E. MacIntyre, L. Lhermitte)
   - Mantle cell lymphoma (MCL-MRD): 1 tube (S. Böttcher)
   - Follicular lymphoma (FL-MRD): 1 tube (S. Böttcher)
   - Marginal zone lymphoma (MZL-MRD): 1 tube (P.Lucio)
   - Lymphoplasmacytic lymphoma (LPL-MRD): 1 tube (P. Lucio)
   - Diffuse large B-cell lymphoma (DLBCL-MRD): 1 tube (P. Lucio)
   - Burkitt lymphoma (BL): 1 tube (L.Lhermitte)
   - T-chronic lymphoproliferative diseases (T-CLPD-MRD): 1 tube (J. Almeida)
   - Multiple myeloma (MM): 1 tube (J. Flores)

Achievement of the EuroFlow Consortium: New concept in diagnostic flow cytometry

EuroFlow

1. Full technical standardization of multicolor flow cytometry (≥8 colors)
   - standardization of instrument settings and laboratory protocols
   - selection of fluorochromes and selection of antibody clones per marker
   - EuroFlow protocols work on all tested ≥8 colors flow cytometers:
     - DAKO Cyan, LSR-II, FACS Canto-II;
     - "late arrivals" (Navios and Gallios): to be tested

2. Implementation and further development of novel software: Infinicyt
   - fast and easy data handling with automated pattern recognition
   - combining multiple tubes: calculation and APS (principle component analysis
   - mapping of diagnosis and follow-up leukemia samples against templates of "normal/control" samples

3. Development of 8-color antibody protocols for diagnosis, classification and monitoring of hematological malignancies
   - 8-color panels are based on recognition of normal cells & differentiation pathways
   - diagnosis and classification tubes are ready; MRD tubes in development
   - flexibility within panels: deletion and inclusion of tubes (and markers) is possible

4. Large EuroFlow data base linked to Infinicyt software

EuroFlow Consortium (LSHB-CT-2006-018708)

Flow cytometry for fast and sensitive diagnosis and follow-up of hematological malignancies

EuroFlow chairman:
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21 laboratories in 14 countries

www.euroflow.org

EuroFlow

Euroflow is an independent scientific consortium,
which aims at innovation in flow cytometry
for improvement of diagnostic patient care
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